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Occurrence of Chicken Anemia Virus in Backyard Chickens of the Metropolitan Region of Belo Horizonte, Minas Gerais

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ABSTRACT

The occurrence of CAV in backyard chickens in the metropolitan area of Belo Horizonte, Brazil, was evaluated. The spleen and thymus of chickens from different origins were collected for DNA extraction and nested-PCR. CAV genome was detected in 30% of the flocks (n=20) examined. CAV origin for backyard chickens is speculated, taking into consideration its widespread incidence in the chicken industry, the contamination of live vaccines with CAV prior to its eradication from SPF flocks, and the use of attenuated CAV vaccines.

INTRODUCTION

Chicken infectious anemia virus (CAV) was described in Brazilian industrially-reared chickens in 1991 (Brentano *et al*., 1991), associated to T-cell and platelet depletion, causing immunosuppression and anemia in young birds. However, considering that no studies have been conducted with backyard or free-range poultry yet, identifying and reducing the numbers and proximity to reservoirs may be important for the control of this disease.

CAV, a virus of genus *Gyrovirus* (Pringle, 1999) and ascribed to Circoviridae family, was first reported as the chicken anemia agent in 1979 in Japan (Yuasa *et al*., 1979; Rosenberger & Cloud, 1998) and described since then in commercial chickens worldwide (Schat, 2003). The virus contains single stranded circular DNA and is highly resistant in the environment (Allan *et al*., 1994), properties which influence its disseminated epidemiology (Todd, 2000). The disease occurs in 10 to 14-day-old old chickens derived from infected breeders, which vertically transmit virus (Yuasa *et al*., 1987). Older chickens do not develop clinical disease, but acquire infection, becoming reservoirs and horizontally transmitting the virus to the progeny (Von Bulow, 1991). Immunosuppression predisposes chickens to opportunistic infections, including bacterial infections, such as necrotic dermatitis, and negatively interferes with vaccination responses (Todd, 2004).

Epidemiological studies carried out in Brazil indicated 92% (Brentano *et al*., 2000), 100% (Canal *et al*., 2004) or 97.2% (Gomes *et al*., 2005) CAV-specific antibody prevalence in broiler breeders after 17 weeks of age. As to free-range or backyard chickens, there is little information. In Nigeria, CAV DNA was found in the sera of 9/12 free-range chickens; however, their nucleotide sequences were different as compared to industrial chickens strains (Oluwayelu & Todd, 2008). In Ecuador, free-range chickens were evaluated (n=100), and CAV-specific antibodies were detected in 90% of the birds (Hernandes-Divers *et al*., 2006).

Backyard or free-range chickens may play a role in the epidemiology of CAV, and the knowledge on their infection status may be important for adopting successful prevention strategies, which are mostly based

on biosecurity and vaccination. CAV infection may be investigated using thymus and spleen sampling, and CAV DNA detection by PCR (Schat, 2003).

An investigation of CAV infection in backyard chickens was conducted. Although CAV was described in the Brazilian poultry industry as already in 1991, and despite the potential importance of backyard chickens in its epidemiology, these birds have not been previously studied. Backyard chickens in the metropolitan area of Belo Horizonte were evaluated using a previously described nested-PCR protocol for CAV DNA detection (Cardona *et al*., 2000), except for silica DNA extraction. Backyard chickens play a significant social-economic role in poor communities, and their flocks are not usually monitored for diseases nor vaccinated. These chickens may be purebred or hybrid, are not fed balanced feeds, and avian pox, avian coriza, mycoplasmosis, Marek's disease, lymphoid leukosis, coccidiosis, and colibacillosis are commonly diagnosed.

MATERIALS AND METHODS

The thymus and spleen of backyard chickens of 20 different origins of Belo Horizonte metropolitan area were collected during 2008, and short-term stored frozen (-20°C) for subsequent DNA extraction. The positive control CAV DNA was extracted from the commercial vaccine AviPro Thymovac (Lohman Animal Health), and the negative control DNA was obtained from SPF embryos (Sadia S.A., Unidade Uberlândia, MG). DNA extraction was performed in pools according to origin and as described by Boom *et al*. (1999), by reaction with sodium iodide (NaI) and DNA absorption of in silica (silicon dioxide, Sigma Chemical Co., St. Louis, MO). Adsorbed DNA in pelleted silica was eluted by adding 50 µl TE (5 mM Tris-HCl pH 8,0, 0,5 mM EDTA pH 8,0), quantified at 260-280nm (NanoDrop ND-1000), and stored frozen (-20ºC) until use as template DNA.

For the nested-PCR, the external primer oligonucleotides O3F 5'-(CAA GTA ATT TCA AAT GAA CG)-3' and O3R 3'-(TTG CCA TCT TAC AGT CTT AT)-5' and internal: N3 5'-(CCA CCC GGA CCA TCA AC)-3' and N4 3'-(GGT CCT CAA GTC CGG CAC ATT C)-5' (Cardona *et al*., 2000) were employed. All reactions provided 50 µl final volumes. The first reaction (external) cycles contained 200 g template DNA, 5 µl 10X buffer (200mM Tris-HCl pH 8,4, 500mM KCl - Invitrogen), 1 µl 10mM dNTP (dATP, dTTP, dCTP e dGTP - Invitrogen), 1,5 µl $MgCl_2$ 50 mM (Invitrogen), 1 µl of each external primer O3F and O3R at 10 mol, 0,2 µl Taq Polimerase at 5U/ l (Platinum® Taq DNA Polymerase - Invitrogen), and ultra pure water q.s.p. Cycles were submitted to initial denaturation at 94ºC/5min, followed by 35 denaturation cycles at 94ºC/1min, anealling at 45ºC/ 2min, and extension at 72ºC/1min, with final extension at 72ºC/10min. The internal reaction (nested-PCR) was performed as described for the external cycles, except for the inclusion of 1 µl of the amplicon product from the first reaction and 10 pmol of the internal primers N3 and N4. CAV DNA negative and template control positive reactions were included in each reaction. The visualization of amplified products, at 388bp for the first reaction, and at 211bp for the second reaction, was performed by electrophoresis in 1% agarose in TBE 0,5X (100mM Tris-base pH 8,3, 25mM EDTA and 50mM boric acid) stained with ethidium bromide, and visualized under an U.V. transiluminator.

RESULTS AND DISCUSSION

Six out of 20 (30%) amplified products (amplicons) presented the expected molecular size for CAV genome fragments, both in the first and the second reactions, as previously described (Cardona *et al*., 2000), at 388bp for the first reaction, and at 211bp in the second reaction, and were considered positive. No amplicon was detected in the negative samples.

All backyard chickens examined presented a health condition, and the presence of CAV infection could have increased the severity of the concurrent disease(s). The most common diseases diagnosed were ectoparasitoses (Mallophaga and Acarina), endoparasitoses (*Ascaridia galli*, *Capillaria* spp., *Eimeria* spp., *Heterakis gallinarum*, *Histomonas meleagridis*, *Raillietina* spp.), mycoses (gastritis by *Macrorhabdus ornithogaster*), and bacterioses (botulism, *Mycoplasma gallisepticum*). However, no correlation could be established between the presence of CAV and health status. When PCR results were compared to the clinical information collected both in positive and negative chickens, no association could be inferred between the gross clinical and pathological examinations and the presence of CAV. The role of CAV as a cause of immunosuppression in hardy unselected chickens is not known. Few studies were conducted with backyard chickens. However, studies indicate high rates of infection. For instance, in Nigeria (Oluwayelu & Todd, 2008), 75% (n=12) of the studied chickens were positive for serum CAV DNA , and 90% (n=100) of chickens evaluated in Ecuador presented CAV-specific antibodies in the serum (Hernandes-Divers *et al*., 2006).

Free-range environments may enable the occurrence of a wide diversity of primary or/and opportunistic pathogens. In addition to the vertically-transmitted agents, the on-site reproduction and chickens of multiple ages also allow inter-generation vertical and horizontal transmission, with perpetuation of certain infections. Most chicken producers purchase free-range chickens from other farmers, continuously adding to the diversity of infectious and parasitic agents. The hardiness of backyard chickens, which is a result of natural challenge selection, however, suggests that they are more resistant to diseases and suffer less impact of CAV infection. In addition, these birds may become healthy reservoirs status, a hypothesis that warrants further studies considering the possibility of the subclinical presentation of chicken anemia.

CAV negative and positive birds both from backyard and industrial flocks were detected in the sampling area, which included the metropolitan region of Belo Horizonte. Industrial chickens have been previously studied in the region, with the detection of CAV DNA and antibodies in our laboratory (data not shown) and a history of discontinued CAV live attenuated strain vaccination (Gomes *et al*., 2005).

The origin of the CAV present in the studied backyard chickens is speculated. Both industrial and SPF chicken flocks were, up to the mid 1990's, vertically infected with CAV (Bülow and Schat, 1997), and therefore industrial chickens (breeders, broilers, layers) could have been a source of CAV for backyard chickens. In addition, the widespread use in chickens of live vaccines produced with CAV-contaminated eggs or cells may have played a role in the dissemination of CAV to backyard chickens due to the previously undetected CAV infection of SPF flocks. Moreover, an attenuated CAV vaccine has been used for a few industrial breeders, and this very resistant virus could have been transferred to backyard chickens. It seems most probable, therefore, that the infection originated in the poultry industry. However, the CAV epidemiology in backyard chickens is unknown. CAV may have gone undetected for years in these flocks in most parts of the world, being a source of infection of industrially-produced chickens.

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